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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07H 15/12, C12N 15/00, 15/13, 5/10, 1/20, C07K 16/08, C12P 19/34, 21/08

A1

(11) International Publication Number: WO 96/16974

(43) International Publication Date: 6 June 1996 (06.06.96)

(21) International Application Number: PCT/US95/15716

(22) International Filing Date: 1 December 1995 (01.12.95)

(30) Priority Data:
08/348,548 1 December 1994 (01.12.94) US

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(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, LS, MW, SD, SZ, UG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PCR AMPLIFICATION OF REARRANGED GENOMIC VARIABLE REGIONS OF IMMUNOGLOBULIN GENES

(57) Abstract

The invention features methods for isolating immunoglobulin variable region genes, and the use of these genes in the production of chimeric and isotype switched antibodies. The invention also features substantially pure DNA encoding a variable region of the antibody produced by hybridoma cell line HNK-20, and chimeric antibodies containing this variable region.

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PCR AMPLIFICATION OF REARRANGED GENOMIC VARIABLE REGIONS OF IMMUNOGLOBULIN GENES

Background of the Invention

The development of mouse hybridoma technology has allowed the production of antibodies (Ab) specific for a wide range of antigens. Mouse monoclonal antibodies (mAb) have been used extensively for diagnosis and, in a few cases, for human therapy and *in vivo* diagnostics. Administration of murine antibodies to humans has been observed to induce a strong human anti-mouse antibody response (HAMA) after single or repeated treatments, thus precluding long-term treatment using these antibodies. Moreover, rodent antibodies are rapidly cleared from human serum and often do not interact effectively with the human immune system. Since human hybridomas are generally unstable and secrete low amounts of antibodies (frequently IgMs), considerable effort has been directed at rendering foreign antibodies (e.g., murine antibodies) more similar to those of the host to which they are administered (e.g., a human). Alternatives to human hybridoma-derived antibodies have been developed in which mouse immunoglobulin sequences (e.g., constant regions) are replaced with corresponding sequences derived from human immunoglobulin genes. Two examples of this type of antibody are (1) chimeric mAbs, in which murine variable regions are combined with human constant regions (Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855, 1984; Boulianne et al., Nature 312:643-646, 1984), and (2) humanized mAbs, in which murine CDRs (complementarity determining regions) replace the corresponding sequences in human immunoglobulins (Jones et al., Nature 321:522-525, 1986; Co et al., Nature 351:501-502, 1991). These engineered antibodies retain their target specificity and generally exhibit reduced

HAMA responses when injected into patients. In addition, desired effector functions of antibodies for certain clinical applications can be obtained by using constant regions corresponding to the appropriate immunoglobulin isotype.

Despite these advances, cloning of variable region sequences has been a limiting step in the rapid construction of chimeric and isotype switched antibodies. Polymerase chain reaction (PCR) amplification of immunoglobulin heavy and light chain variable regions has facilitated this step. However, the high degree of DNA sequence polymorphism in leader and variable sequences of both heavy and light chain genes has required the preparation of complex sets of degenerate primers (Jones et al., Bio/Technology 9:88-89, 1991; Kettleborough et al., Eur. J. Immunol. 23:206-211, 1993; Le Boeuf et al., Gene 82:371-377, 1989; Orlandi et al., Proc. Natl. Acad. Sci. USA 86:3833-3837, 1989). In the case of 5′ primers, these primers have usually been designed to correspond to the first framework of the variable region (FR1) and, in a few cases, to the leader peptide sequence (L). The 3′ primers have usually been designed to correspond to the framework 4 (FR4) region, which displays limited polymorphism, or to the constant region, in which conserved, isotype-specific sequences are easily identified. Although complex sets of 5′ and 3′ primers have been designed, they do not always match the DNA template completely (Gavilondo-Cowley et al., Hybridoma 9:407-417, 1990; Leung et al., BioTechniques 15:286-292, 1993). Native sequences of the immunoglobulin heavy and light chain genes may therefore be altered in the FR1 and/or FR4 regions by the PCR amplification process. Modifications of the N-terminal region of an immunoglobulin, particularly the light chain variable region (VL), in which the amino acid at position two is

part of the predicted canonical structure for CDR1 (Chothia et al., Nature 342:877-883, 1989), have been shown to drastically reduce the affinity of immunoglobulins for their antigens. Moreover, expression levels of the recombinant antibodies may also be altered when mutations occur in the leader peptide. In most studies involving PCR amplification of immunoglobulin H (heavy) and κ/λ (light) chain variable regions using these primers, cDNA templates were used, resulting in the generation of fragments containing incomplete VH and VL sequences, which may or may not be linked to part of the constant region.

Summary of the Invention

We have designed a method for isolating nucleic acids encoding immunoglobulin Fv (variable) fragments from genomic DNA of hybridoma cells producing specific monoclonal antibodies. Specific primers corresponding to (1) the 5′ untranslated region (UTR) of the variable region and (2) the intron downstream of the rearranged JH/Jκ/λ sequences are used in this method. The method can be used to amplify and clone genomic DNA corresponding to λ and κ light chain variable genes, as well as heavy chain variable genes. The variable genes isolated by this method can easily be inserted into expression vectors containing heterologous (e.g., human) light and heavy chain constant genes, thus facilitating isotype switching or antibody chimerization. Using this method, we have cloned for the first time genes encoding the variable regions (Fv) of the kappa light chain and heavy chain of the antibody produced by hybridoma cell line HNK-20.

Accordingly, in one aspect the invention features substantially pure DNA (genomic DNA or cDNA) encoding a variable region of the antibody produced by hybridoma

cell line HNK-20. The variable region can be from the immunoglobulin heavy chain of the antibody, or from the immunoglobulin light chain of the antibody. The DNA may further encode an immunoglobulin constant region, such as a human immunoglobulin constant region. The immunoglobulin can be of any isotype, including, but not limited to an IgA (e.g., IgA1, IgA2, and sIgA), IgG, IgM, IgD, or IgE isotype. In the case of an IgA isotype, the immunoglobulin heavy chain can be an α chain.

In one embodiment, the substantially pure DNA contains the sequence of Fig. 5B, or degenerate variants thereof, and encodes the amino acid sequence of Fig. 5B. In another embodiment, the substantially pure DNA contains a sequence having about 50% or greater sequence identity to the DNA sequence of Fig. 5B. In another embodiment, the substantially pure DNA a) is capable of hybridizing to the DNA sequence of Fig. 5B under stringent conditions; and b) encodes a polypeptide having a biological activity of a HNK-20 variable region.

In another embodiment, the substantially pure DNA contains the sequence of Fig. 5C, or degenerate variants thereof, and encodes the amino acid sequence of Fig. 5C. In another embodiment, the substantially pure DNA contains a sequence having about 50% or greater sequence identity to the DNA sequence of Fig. 5C. In another embodiment, the substantially pure DNA a) is capable of hybridizing to the DNA sequence of Fig. 5C under stringent conditions; and b) encodes a polypeptide having a biological activity of a HNK-20 variable region.

In another embodiment, the substantially pure DNA contains the sequence of Fig. 5D, or degenerate variants thereof, and encodes the amino acid sequence of Fig. 5D. In another embodiment, the substantially pure DNA contains a sequence having about 50% or greater sequence identity to the DNA sequence of Fig. 5D. In another

embodiment, the substantially pure DNA a) is capable of hybridizing to the DNA sequence of Fig. 5D under stringent conditions; and b) encodes a polypeptide having a biological activity of a HNK-20 variable region.

In another aspect of the invention, the DNA is operably linked to regulatory sequences, such as promoter and/or enhancer sequences, for expression of the variable region. In a related aspect, the invention features a vector (e.g., a plasmid or a viral vector) containing the DNA of the invention operably linked to a promoter sequence. The invention also features a cell (e.g., a myeloma cell) containing the DNA of the invention.

In another aspect, the invention features a recombinant antibody containing a variable region from the monoclonal antibody produced by hybridoma cell line HNK-20. In one embodiment, the variable region is from the immunoglobulin heavy chain of the monoclonal antibody. In another embodiment, the variable region is from the immunoglobulin light chain of the monoclonal antibody. The recombinant antibody may further contain a chimeric constant region, e.g., a human immunoglobulin constant region. The antibody of the invention may be of any immunoglobulin isotype, such as those listed above. Accordingly, the antibody of the invention may contain an α heavy chain, and thus be of the IgA isotype.

The invention also features a method of making a recombinant antibody containing a variable region from the monoclonal antibody produced by hybridoma cell line HNK-20. This method involves expression of DNA encoding the variable region of the antibody, as well as a constant region (e.g., a human constant region). Expression of the DNA may be carried out using any standard method known in the art. Preferably, the DNA is expressed in a cell, e.g., a myeloma cell. The cell is cultured under conditions in which the recombinant

antibody is produced, and the antibody is subsequently purified from the cell or from the supernatant in which the cell was cultured, using standard methods.

In a final aspect, the invention features a method of isolating a nucleic acid containing a variable region of an immunoglobulin gene (e.g., the immunoglobulin gene is from hybridoma cell line HNK-20). In this method, a first set of primers, each of which contains the sequence of a polymorphic variant of a segment of the 5′ untranslated region of said immunoglobulin gene; and a second set of primers, each of which contains the sequence of a polymorphic variant of a segment of the intron 3′ to the rearranged J region of said immunoglobulin gene; are used in a polymerase chain reaction containing genomic DNA that encodes the variable region of the immunoglobulin. A set of primers that contain sequences of the immunoglobulin gene is identified from the polymerase chain reaction, and subsequently is used to amplify the immunoglobulin gene.

By "promoter" is meant a minimal sequence element sufficient to direct transcription. Also included in the invention are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type or tissue-specific expression, as well as elements which allow expression to be inducible by external signals or agents; such elements may be located in the 5′ or 3′ regions of, as well as within, the native gene.

By "operably linked" is meant that a gene and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s).

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 50%, preferably 85%, more preferably 90%, and most preferably 95% homology to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 30 nucleotides, preferably at least 50 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

Sequence identity is typically measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705; Ausubel et al., eds. Current Protocols in Molecular Biology, Wiley & Sons, New York, 1989). Such software matches similar sequences by assigning degrees of homology to various substitutions, deletions, substitutions, and other modifications. Conservative substitutions for amino acids typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "substantially pure DNA" is meant DNA that is free of the genes which, in the naturally-occurring genome of the organism from which the DNA is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g.,

a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

"Stringent conditions", as used herein, are defined as follows. High stringency conditions include hybridization at about 42°C in about 50% formamide; a first wash at about 65°C in about 2x SSC and 1% SDS; followed by a second wash at about 65°C in about 0.1% SSC. Lower stringency conditions for genes having about 50% sequence identity include hybridization at about 42°C in the absence of formamide; a first wash at about 42°C in about 6x SSC and about 1% SDS; followed by a second wash at about 50°C in about 6x SSC and about 1% SDS.

Our method for isolating immunoglobulin variable region genes, using genomic DNA templates, does not lead to the production of fragments that need to be adapted for recombinant antibody expression, thus facilitating the generation of chimeric and isotype-switched immunoglobulins. Variable regions with intact coding sequences, including full length leader peptides, are obtained using this method without requiring previous DNA sequencing. Thus, isotype switched mouse Ig and chimeric mouse-human Ig can easily be produced. Chimeric antibodies containing constant regions derived from the host to which the antibody is to be administered (e.g., a human), are advantageous for use in therapeutics, because such chimeric antibodies are less likely than heterologous antibodies (e.g., murine antibodies) to lead to an adverse immune response, e.g., an HAMA response, in the patient.

Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

Detailed Description

The drawings are first described.

Drawings

Figs. 1A-1B are schematic representations of rearranged genes encoding the variable regions of heavy (**Fig. 1A**), kappa (**Fig. 1B**), and lambda (**Fig. 1C**) mouse immunoglobulin chains. Pseudogenes are indicated by "ψ"s. Bold arrows indicate PCR primers corresponding to the 5′ untranslated region and the intron downstream of the rearranged J segment. SalI and NotI restriction sites have been added to the 5′ ends of the PCR primers. Arrows below the J segments represent oligonucleotide probes used in Northern and Southern blot hybridization.

Figs. 2A and 2B are Northern blots of RNA isolated from hybridoma cell lines 3G3 and HNK-20 probed with oligonucleotides homologous to the JH, Jκ, and Jλ segments. **Fig. 2A**: Total RNA (10 μg per slot) from hybridoma cell line 3G3 (IgM, λ) was hybridized with oligonucleotides Jλ1, Jλ2, and Jλ3, as indicated in the figure. **Fig. 2B**: Total RNA (10 μg per slot) from HNK-20 (IgA, κ) was hybridized with Jκ1, Jκ2, Jκ4, and Jκ5; and JH1, JH2, JH3, and JH4 (see Fig. 6 for the sequence of the oligonucleotide probes), as indicated. Oligonucleotides specific for the pseudogenes (Jκ3 and Jλ4) were not used in these experiments. As an internal control for total RNA loading, the blots were rehybridized with a full length glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) cDNA probe (McMaster et al., Proc. Natl. Acad. Sci. USA 74:4835-4838, 1977). Migration positions of ribosomal RNA are indicated.

Figs. 3A-3G are Southern blots of amplified 3G3 Vλ, HNK-20 Vκ, and HNK-20 VH genomic fragments, made by following a two step PCR amplification method. In the first step (**Figs. 3A-3D**), PCR amplification was carried out using groups of 2, 3, or 4, 5′ primers (see Fig. 6)

and a single 3′ primer. The 3′ primer for Vλ was: 3′λ1, for Vκ: 3′κ2, and for VH: 3′H3a or 3′H3b. In the second step (**Figs. 3E-3G**), PCR amplification was performed separately with each of the 5′ primers from the positive groups from the first set of reactions (Figs. 3A-3D), allowing identification of the 5′ primer(s) that generate(s) the signal in each positive group. PCR amplification with 3′H3a was omitted in the second step since 3′H3b generates stronger signals. Positions of size markers of 564, 831, and 947 basepairs are indicated in the lane labelled M1.

Figs. 4A-4B are schematic representations of the rearranged 3G3 Vλ, HNK-20 Vκ, and VH regions. In **Fig. 4A** rearrangement of the lambda locus from 3G3 is shown. In **Fig. 4B** rearrangements of the kappa and heavy chain loci from HNK-20 are shown. Three different kappa chain gene rearrangements have been observed with the Jκ2 segment. For the heavy chain genes, 5′H31a and 5′H31b generate the same PCR product, hence a single rearrangement is observed for the heavy chain. The maps are not drawn to scale.

Figs. 5A-5D are sequences of the PCR amplified 3G3 Vλ, HNK-20 Vκ, and HNK-20 VH regions. The sequences shown in **Figs. 5A**, **5B**, **5C**, and **5D** correspond to 3G3 Vλ (generated by 5′λ1-3′λ1), HNK-20 Vκ (generated by 5′κ16-3′κ2), HNK-20 Vκ (generated by 5′κ30-3′κ2), and HNK-20 VH (generated by 5′H31b-3′H3b), respectively. The sequences of the PCR primers are shown in bold, the CDR regions are underlined, and stop codons are indicated by stars. Regions homologous to the Jλ, Jκ, and JH oligonucleotide probes are double underlined.

Figs. 6A-6F are the nucleotide sequences of the PCR primers and probes used for the amplification of mouse Vλ, Vκ, and VH regions. SalI and NotI sites are in bold, and I represents inosine. Sequences are 5′ to 3′.

The names of the groups of 5′ primers precede the numbering and sequences of the primers.

The Monoclonal Antibody Produced by Hybridoma Cell Line HNK-20.

Respiratory Syncytial Virus (RSV) infection can lead to diseases of the upper or lower respiratory tract, including pneumonia and severe bronchiolitis (McIntosh et al., *In* B. N. Fields and D. M. Knipe (eds.), Virology, Raven Press, New York, pp. 1045-1072, 1990). HNK-20 is a murine hybridoma cell line that produces a monoclonal IgA antibody that recognizes the F glycoprotein of RSV. In addition to neutralizing RSV in plaque reduction assays, this antibody is effective at preventing and treating RSV infection when passively administered to mice intranasally.

Method for Isolating Immunoglobulin Variable Region Genes.

We have designed a strategy for the preparation of genomic fragments encoding the VH-D-JH (immunoglobulin heavy chain variable region) and VL-JL (immunoglobulin light chain variable region) regions of immunoglobulin genes. This strategy involves PCR amplification of genomic DNA prepared from hybridomas using specific primers corresponding to (1) the 5′ untranslated region of the gene encoding the variable region, and (2) an intron downstream from specific rearranged JH/JL sequences. This method does not require previous knowledge of the sequence of any part of the gene encoding the target variable region, thus allowing rapid and efficient isolation of the genes.

Based on analysis of nucleotide sequence databases (e.g., the Genbank), sets of primers are designed corresponding to all of the sequences reported in the

databases for a given organism (e.g., a mouse) for a specific region within the 5′ UTR of the immunoglobulin variable region gene and for a specific region in the intron downstream of the rearranged J segment. In order to minimize the numbers of primers required to cover all of the possible known sequences for a given region (e.g., the 5′ UTR), the most highly conserved specific regions within the region may be focused on. As a specific example, in the case of the 5′ UTR of the murine variable heavy chain, 42 primers are required because of the relatively high degree of nucleotide sequence polymorphism (see below). In contrast, due to lower levels of sequence polymorphism, only 2 primers are required to cover all of the known possibilities for the 5′ UTR of the murine immunoglobulin lambda variable region (see below). In a first set of PCR reactions, multiple primers covering all of the polymorphic sequences corresponding to the region the primers hybridize to can be used in a single reaction. In cases where a large number of primers are required, the primers can be divided up into several reactions based on, e.g., their melting temperatures. For example, for the 5′ UTR of the murine heavy chain variable region, where 42 primers are required to cover all of the known polymorphic sequences, the reactions were divided up into 12 groups (see below). Primers contained in those reactions of the first set of PCR reactions found to be positive can subsequently be tested individually (i.e., in reactions containing only a single 5′ primer and a single 3′ primer) in order to identify the sets of primers that correspond to the sequence of the gene being amplified. These primers can then be used to prepare the fragment for cloning, in order to generate chimeric or isotype switched immunoglobulin genes. As no sequence information for the gene being amplified is required in

the present method, isolating variable region genes from hybridoma cell lines is facilitated.

Variable regions isolated by this method can be fused to genes encoding heterologous constant regions in order to produce chimeric (e.g., a murine variable region fused to a human constant region) or isotype switched immunoglobulin genes, that can in turn be used to make chimeric or isotype switched antibodies. We have used this method to produce chimeric immunoglobulin genes, including one containing the HNK-20 heavy chain variable region fused to a human heavy chain constant region, and another containing the HNK-20 kappa light chain variable region fused to a human kappa light chain constant region. Co-expression of these genes leads to the production of recombinant antibodies having the specificity of the antibody produced by hybridoma cell line HNK-20, and the effector regions of a human antibody.

The recombinant antibodies of the invention can be used to prevent RSV infection, or to treat diseases caused by RSV infection (e.g., upper or lower respiratory tract infections, such as pneumonia or bronchiolitis). The recombinant antibodies of the invention may be administered to any hosts that are susceptible to RSV infection, including, but not limited to, humans (adults, children, and infants), chimpanzees, cattle, cebus monkeys, owl monkeys, ferrets, lambs, mice, rats, and cotton rats. The therapeutic compositions of the invention may be administered to a patient by any appropriate mode. Typically, the antibody is administered to a mucosal surface of the subject, for example, an oral or an intranasal surface. The antibodies of the invention can be administered in an amount determined to be effective for prevention or treatment of RSV infection by one skilled in the art. An

appropriate dosage is one which effects a reduction in the disease caused by RSV infection, and/or one which is effective at preventing RSV infection. It is expected that the dosages will vary, depending upon the mode and route of administration; the age, weight, and health of the recipient; the nature and extent of the disease; the frequency and duration of the treatment; the type, if any, concurrent therapy; and the desired effect. For example, the amount of antibody administered may be in the range of 50 μg/kg to 5 mg/kg body weight. The invention also includes compositions containing the antibodies of the invention in a pharmaceutically acceptable carrier and/or diluent, e.g., saline. Suitable pharmaceutical carriers, as well as pharmaceutical necessities for use in pharmaceutical formulations, are described in *Remington's Pharmaceutical Sciences*, a standard reference text in this field, and in the USP/NF.

The antibodies of the invention may also be used in diagnostic methods for identifying patients infected with RSV, using any of a number of standard assay systems that are well known in the art. Such assay systems include, but are not limited to, enzyme linked immunosorbent assays (ELISA), solid phase radiometric assays, immunofluorescent microscopy, and immunoelectron microscopy (see, e.g., Coligan et al., eds., Current Protocols in Immunology, John Wiley & Sons, New York, 1992). In these methods, an antigen (e.g., from a biological sample, such as cellular material or secretions) is contacted with an antibody of the invention, and specific binding of the antigen and the antibody is detected as a measure of the presence of the antigen in the sample. The detection can be facilitated by the presence of labels on the antibodies of the invention or labels on secondary antibodies that

recognize the antibodies of the invention. The labels that can be employed in these assays include, but are not limited to, radio-labels, enzyme labels (e.g., horse-radish peroxidase), biotin-labels, and chemiluminescent labels, and are detected using standard methods.

Genes encoding immunoglobulin variable regions (both light (kappa and lambda) and heavy chains) isolated using the methods of the present invention can be fused to genes encoding immunoglobulin constant regions (e.g., human constant region genes) in order to make genes encoding chimeric or isotype switched recombinant antibodies (see, e.g., United States Patent Number 4,816,397, issued March 28, 1989; hereby incorporated by reference; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855, 1984; hereby incorporated by reference). For example, in order to generate an IgA antibody, the variable region can be fused to an α constant region. Different types of constant regions that can be used to make the chimeric antibodies of the invention are well known in the art (see, e.g., Roitt et al., eds. Immunology, Gower Medical Publishing, London, 1989), and can be isolated and cloned to make the chimeric and/or isotype switched genes of the invention using standard methods (see, e.g., Sambrook et al., eds. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989; and Coligan et al., *supra*). In order to facilitate cloning of the variable genes into vectors, preferably, the PCR primers used in their amplification contain in their 5' ends sequences recognized by restriction endonucleases. Vectors into which the variable region genes and chimeric genes of the invention can be cloned include both plasmid and viral vectors. Preferred vectors for use in the invention are expression vectors containing appropriate heterologous heavy or light chain

constant genes. For example, genes encoding kappa light chain and heavy chain variable regions can be cloned into plasmids such as pUHWκ and pUHWγ1, respectively (Weissenhorn et al., Gene 106:273-277, 1991, hereby incorporated by reference). These plasmids contain a combination of a heavy-chain enhancer and a μ-gene promoter, as well as a polylinker into which genes encoding variable regions can be inserted. Another vector that can be used in the invention is the pING expression vector (Chomczynski et al., Biochem. Biophys. Res. Commun. 122:340-344, 1984, see below).

The genes of the invention are cloned into the vectors so that the genes are operably linked to appropriate promoter/enhancer sequences. Any promoter that is capable of directing initiation of transcription in a eukaryotic cell may be used in the invention. For example, non-tissue specific promoters, such as the cytomegalovirus (DeBernardi et al., Proc. Natl. Acad. Sci. USA 88:9257-9261, 1991, and references therein), mouse metallothionine I gene (Hammer et al., J. Mol. Appl. Gen. 1:273-288, 1982), HSV thymidine kinase (McKnight, Cell 31:355-365, 1982), and SV40 early (Benoist et al., Nature 290:304-310, 1981) promoters may be used. Preferred promoters for use in the invention include those which direct expression in myeloma cells, as are described above.

Expression of the genes encoding the chimeric and/or isotype switched antibodies of the invention can be achieved using any of a number of methods standard in the art. For example, vectors, e.g., plasmids, containing the HNK-20 variable region(s) fused to heterologous constant regions can be transfected into a cell in which the gene is expressed, either constitutively or upon induction. The genes can be transfected into the cells using any standard method in

the art, including, but not limited to, electroporation, calcium phosphate precipitation, protoplast fusion, and the use of viral vectors or lipids that are coupled with the genes (see, e.g., Sambrook et al., *supra*). Any cell in which the genes of the invention can be expressed to produce the antibodies of the invention may be used. Such cells include, but are not limited to, myeloma cells, such as non-Ig-producing myeloma cells. As a specific example, Sp2/0-Ag14 cells, which are murine myeloma cells that do not secrete or synthesize any immunoglobulin chains, can be used. Sp2/0-Ag14 cells can be obtained from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852-1776, and have been designated ATCC CRL 1581 (see also Ochi et al., Proc. Natl. Acad. Sci. USA 80:6351-6355, 1983). Other cell lines that can be used in the invention include P3X63Ag8.653 (ATCC CRL 1580) and Sp2/01-Ag (ATCC CRL 8006).

The antibodies of the invention can be produced by culturing cells expressing the genes encoding them using standard methods. The antibodies are then purified from the cells and/or cell culture supernatants using standard methods (see, e.g., Coligan et al., *supra*). The culture conditions may be scaled-up using standard methods, in order to generate large quantities of antibody. In addition, the antibodies may be purified from ascites using standard methods.

Experimental Results

Design of the primers and probes for PCR amplification of mouse VH and VL genes.

Most strategies used to amplify mouse VH and VL genes generate fragments encoding variable regions that are either truncated or contain mutations in their 5′ and/or 3′ ends. We have developed a PCR method for

amplifying Fv (variable) genes that results in preservation of the entire Fv sequence. The primers used in this method hybridize to the 5′ untranslated region (the 5′ primers) and in the intron downstream of the rearranged J segment of the lambda, kappa, and heavy chain genes (the 3′ primers; Fig. 1).

DNA sequence comparisons of 5′ untranslated regions and introns downstream from J segments were performed using data from Kabat (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed., U.S. Dept. Health and Human Services, Washington, DC, 1991) and the Genbank and European Molecular Biology Laboratory (EMBL) libraries, and were further analyzed with the Genetics Computer Group (GCG, Madison, WI) package programs. Alignment of the DNA sequences of the 5′ untranslated region revealed that the polymorphism in this region is globally identical to that of the leader peptide sequences. However, a stretch of around 20 nucleotides immediately upstream of the start codon (ATG) was observed to be more conserved throughout the alignment. We therefore designed a series of 5′ PCR primers (18-mers) that terminate with the ATG sequence, ensuring perfect matches of the 3′ ends of each primer with the template, as is critical for initiation of consistent priming by Taq polymerase. A SalI restriction site and 4 additional nucleotides, which facilitate digestion by the restriction endonuclease, were added to the 5′ end of the primers in order to expedite cloning of the PCR fragments. Complex sets of 5′ primers were synthesized consisting of 2 primers for lambda, 30 primers for kappa, and 42 primers for the heavy chain genes, some of which primers contained inosine residues, or were degenerate (Fig. 6).

In contrast, alignment of the 5′ end of the intron sequences downstream from the J segments did not reveal

significant polymorphism, so that 3′ PCR primers specific for each of the Jλ, Jκ, and JH segments were designed in this intronic region. Single PCR primers were prepared corresponding to the regions downstream from each J segment, except for the JH3 segment, for which 2 primers were needed because of DNA sequence polymorphism. The 3′ PCR primers are 18-mers, except for the primer downstream of the JH1 segment which was designed as a 20-mer because of the high A/T content of this region. A NotI restriction site and 4 additional nucleotides were added to the 5′ end of these oligonucleotides in order to allow directional cloning of the PCR fragments into expression vectors (Fig. 6).

In order to ensure specificity of the PCR amplifications and to better characterize the VH-D-JH and VL-JL rearrangements, JL and JH-specific oligonucleotide probes were designed. Sets of oligonucleotides (19-mers) specific for each of the Jλ, Jκ, and JH segments, which exhibit very low polymorphism, were synthesized. These oligonucleotides were used as probes in both Southern blot analysis of the PCR fragments, and Northern blot analysis of total RNA extracted from mouse hybridoma cells.

Specificity of the oligonucleotide probes.

The specificity of the oligonucleotide probes homologous to each J segment in DNA-DNA hybridization was demonstrated by Southern blot hybridization using a variety of cloned VL and VH PCR fragments of known sequences. The specificity of these probes was also tested by hybridization to RNA on Northern blots. RNA from the hybridoma cells 3G3 (IgM, λ) and HNK-20 (IgA, κ) were hybridized with the Jλ, Jκ, and JH oligonucleotides (Fig. 2). The Northern blot analysis revealed that probes Jλ1, Jκ2, and JH3 generate signals at the expected sizes for 3G3 and HNK-20, respectively. Hybridization of

HNK-20 RNA with the Jκ2 probe generated a much stronger signal than the JH3 probe, although these two probes had similar specific activities and identical calculated melting temperatures. This observation suggests either that the kappa chain transcript is much more abundant, or that several kappa chain transcripts hybridize with the Jκ2 probe. PCR amplification of the Vκ region supports the hypothesis that there are several different kappa chain gene rearrangements, one of which was derived from a transcribed pseudogene rearranged with the Jκ2 segment present in the immortalized fusion partner X63Ag8.653 (Strohal et al., Nucleic Acids Res. 15:2771, 1987; Carroll et al., Mol. Immunol. 25:991-995, 1988). Thus, at least one kappa pseudogene and one kappa functional gene transcript, both rearranged with the Jκ2 segment, are contributing to the strong signal observed. The Northern blots were then rehybridized with a probe specific for the house-keeping enzyme gene glyceraldehyde-3-phosphate-dehydrogenase (Piechaczyk et al., Nucleic Acids Res. 12:6951-6963, 1984) to control for the amount of RNA loaded (Fig. 2).

Use of the above-described J-specific oligonucleotide probes allows rapid characterization of the PCR products and immunoglobulin gene transcripts, and further allows identification of the J segment used in the rearrangement of the light and heavy chain genes.

Amplification and detection of mAb variable region genes.

Several immunoglobulin variable region genes have been amplified by PCR using genomic DNA prepared from mouse hybridoma cells. As specific examples, amplification of the Vλ gene of the hybridoma cell line 3G3, and the Vκ gene and VH genes of hybridoma cell line HNK-20 are described.

Based on the Northern blot analysis shown in Fig. 2, it was established that the λ chain gene was rearranged with Jλ1, the κ chain gene was rearranged with Jκ2, and the heavy chain gene was rearranged with JH3. Therefore, the 3′ primers required for amplification were 3′λ1, 3′κ2, and 3′H3a/3′H3b. Due to the high level of polymorphism in the 5′ untranslated region, if all of the 5′ primers were used in separate PCR reactions, there would be 2 PCR reactions for lambda, 30 PCR reactions for kappa, and 42 PCR reactions for the heavy chain genes. To reduce this complexity, the 5′ primers were divided into small groups of 2, 3, or 4 primers based on their melting temperatures (Fig. 6). One group was required for λ, 9 for κ, and 12 for the heavy chain gene primers, reducing the number of reactions to 1, 9, and 12, respectively.

The fragments produced in the first set of PCR amplification reactions, as analyzed on Southern blots hybridized with the Jλ1, Jκ2, and JH3 probes, corresponded to the expected sizes in the 5′λ group a, in the 5′κ groups g and i, and in the 5′H groups c and l (Figs. 3A, 3B, 3C, and 3D). In the second set of PCR reactions, each 5′ primer from the positive groups identified in the first set of PCR reactions was combined with the corresponding 3′ primer, and the amplified fragments were analyzed on Southern blots using the Jλ1, Jκ2, and JH3 oligonucleotide probes. This experiment revealed that 5′λ1, 5′κ30, 5′κ16, 5′κ19 and 5′H31B generate the predicted signals (Figs. 3E, 3F, and 3G), indicating that rearranged fragments were found for one lambda chain gene in 3G3, 3 kappa chain genes in HNK-20, and one heavy chain gene in HNK-20 (Fig. 4). Using another mouse hybridoma cell (PCG-4), secreting IgG2a with a κ chain, 3 different rearrangements of the kappa chain genes, and a single rearrangement of the heavy

chain gene, were observed using the same PCR strategy. This observation is consistent with the presence of both a functional Vκ and aberrant Vκ transcripts in hybridoma cells obtained by fusion using cell lines derived from the original MOPC-21 tumor (Strohal et al., Nucleic Acids Res. 15:2771, 1987; Carroll et al., Mol. Immunol. 25:991-995, 1988). A third non-transcribed kappa chain rearrangement was detected in both HNK-20 and PCG-4 hybridomas which involved different Jκ segments. Since the only κ-chain gene that the fusion partner can contribute is the non-functional MOPC-21 κ gene (Storb et al., Nucleic Acids Res. 8:4681-4687, 1980), this third rearrangement probably originated from the mouse B-cell that served as a fusion partner.

Sequence analysis of the Vλ, Vκ, and VH PCR fragments.

The PCR amplified variable regions of 3G3 Vλ, HNK-20 Vκ, the HNK-20 Vκ pseudogene, and HNK-20 VH were each cloned into the pING expression vector (Chomczynski et al., *supra*) and sequenced using the dideoxy chain termination method (Piechaczyk et al., Nucleic Acids Res. 12:6951-6963, 1984). The corresponding sequences are shown in Figs. 5A-5D. The deduced amino acid sequences of the Vλ, Vκ, and VH fragments correspond to open reading frames consistent with those reported in the various gene banks. In all three PCR amplified fragments, the positions of the cysteines involved in intramolecular disulfide bridge formation were conserved. The presence of conserved stretches of amino acids corresponding to framework sequences allowed positioning of the CDRs (Fig. 5). Taken together, these observed structural features indicate that the amplified and cloned Fv fragments are functional and thus are able to recognize their corresponding antigen, provided they are expressed in appropriate host cells. Accordingly, the murine κ and H Fv genes corresponding to the antibody

produced by the HNK-20 hybridoma have been inserted into expression vectors containing human heavy and light chain constant genes and transfected into myeloma cells.

Materials and Methods

Sources of DNA and RNA.

Mouse hybridoma cell line 3G3 (IgM, λ) was deposited with the American Type Culture Collection, and was assigned ATCC No. HB 8516. Mouse hybridoma cell line HNK-20 (IgA, κ), from OraVax Inc., Cambridge, USA, was deposited with the American Type Culture Collection (ATCC, Rockville, Maryland) under the provisions of the Budapest Treaty on July 1, 1993, and was assigned ATCC No. HB 11394. Genomic DNA was prepared as described (Gross-Bellard et al., Eur. J. Biochem. 36:32-38, 1973, hereby incorporated by reference). Total RNA was prepared from frozen cell pellets according to the guanidine/cesium chloride method (Glisin et al., Biochemistry 13:2633-2637, 1974; Chirgwin et al., Biochemistry 18:5294-5299, 1979).

Synthesis of oligonucleotide primers and probes.

Oligonucleotides were synthesized using standard methods involving the β-cyanoethyl phosphoramidite method and reverse phase HPLC purification (MWG-Biotech, Ebersberg, Germany).

DNA amplification by PCR.

In vitro DNA amplification (PCR) was performed in a final volume of 100 µl volume using the thermal cycler 9600 from Perkin Elmer (Norwalk, CT). Reagents were added to the reaction to yield the following final composition: 10 mM Tris-HCl pH 8.3 (at 25°C), 50 mM KCl, 2.5 mM $MgCl_2$, 0.001% gelatin (Sigma, Cat. No. G2500, St. Louis, MO), 200 µM dNTPs, 150 nM of each amplification primer, 1 µg of genomic DNA, and 2.5 units of AmpliTaq DNA polymerase (Perkin Elmer, Norwalk, CT). The cycling profile used is as follows: 5 minutes at 94°C; 3 cycles

of 1 minute at 94°C, 1 minute at 50°C, and 1 minute at 72°C; 27 cycles of 1 minute at 94°C, 1 minute at 62°C, 1 minute at 72°C; and a final incubation at 72°C for 10 minutes.

Northern and Southern blot analysis.

Total RNA (10 μg) was denatured with glyoxal and fractionated by electrophoresis on an agarose gel (McMaster et al., Proc. Natl. Acad. Sci. USA 74:4835-4838, 1977). Transfer of RNA onto Gene Screen Plus membrane (Dupont, Wilmington, DE) was carried out according to the procedure described by the manufacturer. PCR products and genomic DNA prepared from hybridoma cells were fractionated on agarose gels and transferred onto Gene Screen Plus membrane as described (Chomczynski et al., Biochem. Biophys. Res. Commun. 122:340-344, 1984).

Northern and Southern blots were hybridized in identical conditions with ^{32}P-labeled oligonucleotide probes specific for each of the Jλ, Jκ, and JH segments. The temperatures used for prehybridization and hybridization were TM-4°C, while the temperatures used for washing were TM-2°C. The melting temperatures (TMs) of the oligonucleotides were estimated by the formula: TM = 4(G+C) + 2(A+T). The TMs of the J-specific oligonucleotide probes are as follows: Jλ1 = 58°C, Jλ2 = 62°C, Jλ3 = 60°C, Jλ4 = 58°C, Jκ1 = 64°C, Jκ2 = 64°C, Jκ3 = 56°C, Jκ4 = 64°C, Jκ5 = 62°C, JH1 = 68°C, JH2 = 64°C, JH3 = 64°C, and JH4 = 60°C. For both Southern and Northern blots probed with oligonucleotide probes, the following conditions were used. Prehybridization was carried out for 3-4 hours in 2x SSC, 5x Denhardts, 0.1% SDS, and 5 mM EDTA. Hybridization was carried out for 14-18 hours in 5x SSC, 10x Denhardts, 20 mM sodium phosphate buffer (pH 7.0), 7% SDS, 100 μg/ml denatured herring sperm DNA, and ^{32}P-labeled oligonucleotide (106

cpm/ml). After hybridization, the membranes were washed twice for 30 minutes in 3x SSC, 10x Denhardts, 5% SDS, and 70 mM sodium phosphate buffer (pH 7.0); and twice for 30 minutes in 1x SSC and 1% SDS.

As an internal control for total RNA loading, Northern blots were hybridized with a glyceraldehyde-3-phosphate-dehydrogenase cDNA probe (Piechaczyk et al., Nucleic Acids Res. 12:6951-6963, 1984). For both Northern and Southern blots probed with the cDNA probe, the following conditions were used. Prehybridization was carried out for 3-5 hours at 42°C in 50% deionized formamide, 5x Denhardts, 5x SSPE (30x SSPE = 4.5 M NaCl, 0.3 M NaH_2PO_4, 30 mM EDTA, pH 7.7), 1% SDS, and 200 μg/ml denatured salmon sperm DNA. Hybridization was carried out for 14-18 hours at 42°C in the same buffer including ^{32}P-labeled cDNA probe (106 cpm/ml). After hybridization, the membranes were washed twice for 5 minutes in 2x SSPE at room temperature, once for 15 minutes in 2x SSPE and 0.5% SDS at 65°C, and once for 15 minutes in 0.5x SSPE at 65°C.

DNA sequencing.

DNA sequences were determined by subcloning the L-VH-D-JH and L-VL-JL PCR fragments in the expression vector pING (Liu et al., Gene 54:33-40, 1987), followed by sequencing using the dideoxy chain termination method (Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463-5467, 1977).

Other Embodiments

The invention also includes any biologically active fragment or analog of the genes and antibodies of the invention. By "biologically active" is meant possessing any activity which is characteristic of HNK-20 variable genes or chimeric antibodies containing them. The invention also includes analogs of the HNK-20

immunoglobulin variable chains. Preferred analogs include those with sequences which differ from the sequences shown in Figs. 5B-5D only by conservative amino acid substitutions, for example, substitution of one amino acid for another with similar characteristics (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions which do not abolish the immunoglobulin's biological activity. Analogs of the invention will generally exhibit at least 70%, preferably 80%, more preferably 90%, and most preferably 95%, or even 99%, homology with a segment of 20 amino acid residues, preferably 40 amino acid residues, or more preferably the entire sequence of an immunoglobulin of the invention. Alterations in the primary sequence include genetic variants, both natural or induced. Also included are analogs that include residues other than naturally occurring or synthetic amino acids, e.g., β or γ amino acids. Also included are immunoglobulins modified by *in vivo* chemical derivitization, including acetylation, methylation, phosphorylation, carboxylation, or glycosylation.

In addition to substantially full-length polypeptides, the invention also includes biologically active fragments of the immunoglobulins, which can be made using standard methods in the art. As used herein, the term "fragment," as applied to a polypeptide, such as an immunoglobulin, will ordinarily be at least 20 residues, more preferably at least 40 residues in length. Similarly, the invention also includes fragments of the genes encoding the immunoglobulins of the invention.

It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are

to be included within the spirit and scope of this application and the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Kraehenbuhl, Jean Pierre

(ii) TITLE OF INVENTION: PCR AMPLIFICATION OF REARRANGED GENOMIC VARIABLE REGIONS OF IMMUNOGLOBULIN GENES

(iii) NUMBER OF SEQUENCES: 108

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30B

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/348,548
(B) FILING DATE: 01-DEC-1994

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(C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 537 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GATCGTCGAC CGTGGTTTGT GAATTATGGC CTGGATTTCA CTTATACTCT CTCTCCTGGC 60

TCTCAGCTCA GGTCAGCAGC CTTTCTACAC TGCAGTGGGT ATGCAACAAT GCGCATCTTG 120

TCTCTGATTT GCTACTGATG ACTGGATTTC TCATCTGTTT GCAGGGGCCA TTTCCCAGGC 180

TGTTGTGACT CAGGAATCTG CACTCACCAC ATCACCTGGT GAAACAGTCA CACTCACTTG 240

TCGCTCAAGT ACTGGGGCTG TTACAACTAG TAACTATGCC AACTGGGTCC AAGAAAAACC 300

AGATCATTTA TTCACTGGTC TAATAGGTGG TACCAACAAC CGAGCTCCAG GTGTTCCTGC 360

CAGATTCTCA GGCTCCCTGA TTGGAGACAA GGCTGCCCTC ACCATCACAG GGGCACAGAC 420

TGAGGATGAG GCAACATATT TCTGTGCTCT ATGGTACAGC AACCATTGGG TGTTCGGTGG 480

AGGAACCAAA CTGACTGTCC TAGGTGAGTC ACTGGTCCCT CCTTTGCGGC CGCTGAT 537

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Trp Ile Ser Leu Ile Leu Ser Leu Leu Ala Leu Ser Ser Gly
1 5 10 15

Ala Ile Ser Gln Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser
20 25 30

Pro Gly Glu Thr Val Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val
35 40 45

Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln Glu Lys Pro Asp His Leu
50 55 60

Phe Thr Gly Leu Ile Gly Gly Thr Asn Asn Arg Ala Pro Gly Val Pro
65 70 75 80

Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile
85 90 95

Thr Gly Ala Gln Thr Glu Asp Glu Ala Thr Tyr Phe Cys Ala Leu Trp
100 105 110

Tyr Ser Asn His Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
115 120 125

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 554 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATCGTCGAC GGACTCAGCA TGGACATGAG GACCCCTGCT CAGTTTCTTG GAATCTTGTT 60

GCTCTGGTTT CCAGGTAAAA TGAACTAAAA TGGGAATGTC ACTGTGATTA GTGTTGATTG 120

GCATTTGGGA GATTTTATCT TTTATGATGC TTACCTATGT AGATACTCAT TATGTCTCCA 180

TTCCTAGGTA TCAAATGTGA CATCAAGGTG ACCCAGTCTC CATCTTCCAT GTATGCATCT 240

CTAGGAGAGA GAGTCACTAT CACTTGCAAG GCGAGTCAGG ACATTAATAA CTATTTAAAC 300

TGGTTCCAGC AGAAACCAGG GAAATCTCCT AAGACCCTGA TCTATCGTGC AAACAGATTG 360

CTAGATGGGG TCCCATCAAG GTTCAGTGGC AGTGGATCTG GGCAAGATTA TTCTCTCACC 420

ATCAGCAGCC TGGAGTATGA AGATATGGGA ATTTATTATT GTCTACAGTT TGACGAGTTT 480

CCGTACACGT TCGGAGGGGG GACCAAGCTG GAAATAAAAC GTAAGTAGTC TTCTCAACTC 540

TTGCGGCCGC TGAT 554

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 127 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Arg Thr Pro Ala Gln Phe Leu Gly Ile Leu Leu Leu Trp Phe Pro
1 5 10 15

Gly Ile Lys Cys Asp Ile Lys Val Thr Gln Ser Pro Ser Ser Met Tyr
20 25 30

Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
35 40 45

Ile Asn Asn Tyr Leu Asn Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro
50 55 60

Lys Thr Leu Ile Tyr Arg Ala Asn Arg Leu Leu Asp Gly Val Pro Ser
65 70 75 80

Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
85 90 95

Ser Leu Glu Tyr Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Phe Asp
100 105 110

Glu Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
115 120 125

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 690 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATCGTCGAC TTCCAGCTCT CAGAGATGGA GACAGACACA CTCCTGTTAT GGGTACTGCT 60

GCTCTGGGTT CCAGGTGAGA GTGCAGAGAA GTGTTGGATG CAACCTCTGT GGCCATTATG 120

ATACTCCATG CCTCTCTGTT CTTGATCACT ATAATTAGGG CATTTGTCAC TGGTTTTAAG 180

TTTCCCCAGT CCCCTGAATT TTCCATTTCC TCAGAGTGAT GTCCAAAATT CTTCTTAAAA 240

ATTTAAATCA AAAGGTCCTC TGCTGTGAAG TCTTTTATAC ATATATAACA ATAATCTTTG 300

TGTTTATCAT TCCAGGTTCC ACTGGTGACA TTGTGCTGAC ACAGTCTCCT GCTTCCTTAG 360

CTGTATCTCT GGGGCAGAGG GCCACCATCT CATACAGGGC CAGCAAAAGT GTCAGTACAT 420

CTGGCTATAG TTATATGGCG TGGAACCAAC AGAAACCAGG ACAGCCACCC AGACTCCTCA 480

TCTATCTTGT ATCCAACCTA GAATCTGGGG TCCCTGCCAG GTTCAGTGGC AGTGGGTCTG 540

GGACAGACTT CACCCTCAAC ATCCATCCTG TGGAGGAGGA GGATGCTGCA ACCTATTACT 600

GTCAGCACAT TAGGGAGCTT ACACGTTCGG AGGGGGGACC AAGCTGGAAA TAAAACGTAA 660

GTAGTCTTCT CAACTCTTGC GGCCGCTGAT 690

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
20 25 30

Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Tyr Arg Ala Ser Lys Ser
35 40 45

Val Ser Thr Ser Gly Tyr Ser Tyr Met Ala Trp Asn Gln Gln Lys Pro
50 55 60

Gly Gln Pro Pro Arg Leu Leu Ile Tyr Leu Val Ser Asn Leu Glu Ser
65 70 75 80

Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys
100 105 110

Gln His Ile Arg Glu Leu Thr Arg Ser Glu Gly Gly Pro Ser Trp Lys
115 120 125

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 546 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATCGTCGAC CTCAAGGTCC TTACAATGAA ATGCAGCTGG GTCATCTTCT TCCTGATGGC 60

AGTGGTTACA GGTAAGGAGC TCCCAAGTCC CAAACTTGAG GGGCCATACA CTCTGTGACA 120

GTGGCAGTCA CTTTGCCTTT CTTTCTACAG GGGTCAATTC AGAGGTTCAG CTGCAGCAGT 180

CTGGGGCTGA GCTTGTGAGG CCAGGGGCCT TAGTCAAGTT GTCCTGCAAA GCCTCTGGCT 240

TCAACATTAA AGACTACTAT ATGTACTGGG TAAAACAGAG GCCTGAACAG GGCCTGGAGT 300

GGATTGGATG GATTGATCCT GAAAATGGTA ATACTGTTTA TGACCCGAAG TTCCAGGGCA 360

AGGCCAGTAT AACAGCAGAC ACATCCTCCA ACACAGCCTA CCTGCAGCTC AGCAGCCTGG 420

CATCTGAGGA CACTGCCGTC TATTACTGTG CTTACTACGG TACTAGCTAC TGGTTTCCTT 480

ACTGGGGCCA AGGGACTCTG GTCACTGTCT CTGCAGGTGA GTCCTACCTT CTCCGCGGCC 540

GCTGAT 546

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 136 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Thr Val
1 5 10 15

Asn Ser Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro
20 25 30

Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys
35 40 45

Asp Tyr Tyr Met Tyr Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu
50 55 60

Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asn Thr Val Tyr Asp Pro
65 70 75 80

Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr Ser Ser Asn Thr
85 90 95

Ala Tyr Leu Gln Leu Ser Ser Leu Ala Ser Glu Asp Thr Ala Val Tyr
100 105 110

Tyr Cys Ala Tyr Tyr Gly Thr Ser Tyr Trp Phe Pro Tyr Trp Gly Gln
115 120 125

Gly Thr Leu Val Thr Val Ser Ala
130 135

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GATCGTCGAC CTTGGTTTGT GAATTATG 28

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GATCGTCGAC AGTAGTACCT GCATTATG 28

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GATCGCGGCC GCAAAGGAGG AGGAGTTAC 29

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATCAGCGGCC GCAAGAAGCA TTAAAGCCAC 30

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATCAGCGGCC GCAAGAAGCT TGAAACTAC 30

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GATCGTCGAC AAATTCAAAK ACAMAAT 27

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GATCGTCGAC AAGACTCAGC CTGACATG 28

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GATCGTCGAC AAGTTCAAAG ACAAAATG 28

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GATCGTCGAC AGACTCAGCC TGACATG 27

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GATCGTCGAC AGCAGGGGGA GCAGGATG 28

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GATCGTCGAC AGGGAAAGTT TGAAGATG 28

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GATCGTCGAC ATACATCAGA CCAGCATG 28

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GATCGTCGAC ATCTAGYTCT CAGAGATG 28

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GATCGTCGAC ATGCATCACA CCAGCATG 28

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GATCGTCGAC CACCAAGTTC TCAGAATG 28

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GATCGTCGAC CAGAGCAGCA GGGACATG 28

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GATCGTCGAC CAGGGACAAG TGGGAATG 28

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GATCGTCGAC CATTCAGAAC TCAGCATG 28

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GATCGTCGAC GCGAGTCAGA CCAGCATG 28

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GATCGTCGAC GGACACAGTT TAGATATG 28

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GATCGTCGAC GGACTCAGCA TGGACATG 28

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GATCGTCGAC GGAGACGTTG TAGAAATG 28

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GATCGTCGAC GGATACACCA TCAGCATG 28

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GATCGTCGAC GGCAARGGCA TCAAGATG 28

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GATCGTCGAC GGCAGKGGRA GCAAGAT 27

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GATCGTCGAC GGTCACAGCA CAAACATG 28

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GATCGTCGAC GGTTGCCTCC TCAAAATG 28

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GATCGTCGAC GTTCATTTCC TCAAAATG 28

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GATCGTCGAC TATCAAGTTC TCAGAATG 28

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GATCGTCGAC TCTCAAGTTC TCAGAATG 28

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GATCGTCGAC TCTTGTGAAT TAATCATG 28

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GATCGTCGAC TGAAAACACA CAGACATG 28

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GATCGTCGAC TGATAAAGCC AAGGAATG 28

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GATCGTCGAC TGATCACACA CAGWCATG 28

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GATCGTCGAC TTCCAGCTCT CAGAGATG 28

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

ATCAGCGGCC GCAGAGASTT TGGATTCTAC 30

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

ATCAGCGGCC GCAAGAGTTG AGAAGACTAC 30

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

ATCAGCGGCC GCAGTTGAGC AAAAATGTAC 30

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

ATCAGCGGCC GCAAATGAGC AAAARTCTAC 30

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ATCAGCGGCC GCAAGATGAG AAAAGTGTAC 30

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GATCGTCGAC ACACAGACTC ACACCATG 28

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GATCGTCGAC ACACAGGACC TCACCATG 28

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GATCGTCGAC ACACAGGATC TCACCATG 28

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GATCGTCGAC ACACAGGGCA TTGCCATG 28

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GATCGTCGAC ACACTGACTC AAAACATG 28

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GATCGTCGAC ACACTGACTC AAACCATG 28

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GATCGTCGAC ACACTGACTC ACACCATG 28

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GATCGTCGAC ACACTGACTC CAACCATG 28

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GATCGTCGAC ACACTGACTC TAACCATG 28

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GATCGTCGAC ACACTGACTC TCACCATG 28

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GATCGTCGAC ACACTGACTT CACCATG 27

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GATCGTCGAC ACATAGACTC TAACCATG 28

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GATCGTCGAC ACATTGACTC AAACCATG 28

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GATCGTCGAC AGCCTCCATC AGAGCATG 28

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GATCGTCGAC AGCCTCCGTC AGAGCATG 28

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GATCGTCGAC ATTATAACAT TGAACATG 28

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GATCGTCGAC CAAGTCTTAG ACATCATG 28

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GATCGTCGAC CACACATCCC TTACCATG 28

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GATCGTCGAC CACAGACACC TCACCATG 28

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GATCGTCGAC CACAGACCMC TCACCATG 28

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GATCGTCGAC CACAGACCTG TCAACATG 28

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

GATCGTCGAC CACAGACCTG TCACCATG 28

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GATCGTCGAC CACGGAACCC TCACCATG 28

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

GATCGTCGAC CACGGACCCC TCACCATG 28

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

GATCGTCGAC CACGGACCCC TCACGATG 28

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

GATCGTCGAC CACTCGACTC TAACCATG 28

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GATCGTCGAC CACTGGTGTG CAGTCATG 28

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

GATCGTCGAC CACTTCTTAG ACATCATG 28

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GATCGTCGAC CAGAGTCCAC TCRCCATG 28

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GATCGTCGAC CCTGTCACTG ACTTCATG 28

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GATCGTCGAC CTCAAGGTCC TTACAATG 28

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GATCGTCGAC CTCCAGGTCC TTACAATG 28

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GATCGTCGAC CTCAGTCCTG TCACCATG 28

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GATCGTCGAC CTCAGTCCTG TCACTATG 28

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GATCGTCGAC GCAGAGGACC TCACAATG 28

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GATCGTCGAC GCCTTTACAG ACTTCATG 28

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

GATCGTCGAC GGACCTCACC ATGGGATG 28

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GATCGTCGAC GGGTGTTGCC TAAGGATG 28

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GATCGTCGAC GGTGTWGCCT AAAAGATG 28

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

GATCGTCGAC GGTGTTGCCT AAAGGATG 28

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

GATCGTCGAC GTTGTAGCCT AAAAGATG 28

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GATCGTCGAC TCAGTCCTTG TCACTATG 28

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

ATCAGCGGCC GCAAAGAAAA AAGCCAGCTT AC 32

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

ATCAGCGGCC GCGAGGTTKT AAGGACTCAC 30

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

ATCAGCGGCC GCGGAGAART TAGGACTCAC 30

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

ATCAGCGGCC GCGGAGAAGK TAGGACTCAC 30

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

ATCAGCGGCC GCTGGAGAGG CCATTCTTAC 30

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GTCAGTTTGG TTCCTCCAC 19

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

GTGACCTTGG TTCCACCGC 19

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

GTGACCTTGG TTCCACTGC 19

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

GTCAATCTGG TTCCACCTC 19

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

GTGCCTCCAC CGAACGTCC 19

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

GTCCCCCCTC CGAACGTGT 19

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

GTCCCATCAC TGAATGTGA 19

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

GTCCCCGAGC CGAACGTGA 19

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

GTCCCAGCAC CGAACGTGA 19

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

GACCGTGGTC CCTGCGCCC 19

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

GAGAGTGGTG CCTTGGCCC 19

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

GACCAGAGTC CCTTGGCCC 19

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

GACTGAGGTT CCTTGACCC 19

What is claimed is:

1. Substantially pure DNA encoding a variable region of the antibody produced by hybridoma cell line HNK-20.

2. The DNA of claim 1, wherein said variable region is from the immunoglobulin heavy chain of said antibody.

3. The DNA of claim 1, wherein said variable region is from the immunoglobulin light chain of said antibody.

4. The DNA of claim 1, further encoding an immunoglobulin constant region.

5. The DNA of claim 4, wherein said immunoglobulin constant region is a human immunoglobulin constant region.

6. The DNA of claim 4, wherein said DNA encodes an immunoglobulin α heavy chain.

7. The DNA of claim 1, wherein said DNA is genomic DNA.

8. The DNA of claim 1, wherein said DNA is cDNA.

9. Substantially pure DNA comprising the sequence of Fig. 5B, or degenerate variants thereof, and encoding the amino acid sequence of Fig. 5B.

10. Substantially pure DNA comprising a sequence having about 50% or greater sequence identity to the DNA sequence of Fig. 5B.

11. Substantially pure DNA which a) is capable of hybridizing to the DNA sequence of Fig. 5B under stringent conditions; and b) encodes a polypeptide having a biological activity of a HNK-20 variable region.

12. Substantially pure DNA comprising the sequence of Fig. 5C, or degenerate variants thereof, and encoding the amino acid sequence of Fig. 5C.

13. Substantially pure DNA comprising a sequence having about 50% or greater sequence identity to the DNA sequence of Fig. 5C.

14. Substantially pure DNA which a) is capable of hybridizing to the DNA sequence of Fig. 5C under stringent conditions; and b) encodes a polypeptide having a biological activity of a HNK-20 variable region.

15. Substantially pure DNA comprising the sequence of Fig. 5D, or degenerate variants thereof, and encoding the amino acid sequence of Fig. 5D.

16. Substantially pure DNA comprising a sequence having about 50% or greater sequence identity to the DNA sequence of Fig. 5D.

17. Substantially pure DNA which a) is capable of hybridizing to the DNA sequence of Fig. 5D under stringent conditions; and b) encodes a polypeptide having a biological activity of a HNK-20 variable region.

18. The DNA of claim 1, wherein said DNA is operably linked to regulatory sequences for expression of said variable region; and

wherein said regulatory sequences comprise a promoter.

19. A vector comprising the DNA of claim 1.

20. A cell comprising the DNA of claim 1.

21. A recombinant antibody comprising a variable region from the monoclonal antibody produced by hybridoma cell line HNK-20.

22. The recombinant antibody of claim 21, wherein said variable region is from the immunoglobulin heavy chain of said monoclonal antibody.

23. The recombinant antibody of claim 21, wherein said variable region is from the immunoglobulin light chain of said monoclonal antibody.

24. The recombinant antibody of claim 21, further comprising a human immunoglobulin constant region.

25. The recombinant antibody of claim 21, further comprising an immunoglobulin α heavy chain.

26. A method of making a recombinant antibody comprising a variable region from the monoclonal antibody produced by hybridoma cell line HNK-20, said method comprising the steps of:

a. providing DNA encoding said variable region of said monoclonal antibody, said DNA further encoding a constant region;

b. expressing said DNA.

27. A method of making a recombinant antibody comprising a variable region from the monoclonal antibody produced by hybridoma cell line HNK-20, said method comprising the steps of:

a. providing a cell containing DNA encoding said variable region of said monoclonal antibody, said DNA further encoding a constant region;

b. culturing said cell under conditions in which said recombinant antibody is produced; and

c. purifying said recombinant antibody from said cell or from the supernatant in which said cell was cultured.

28. A method of isolating a nucleic acid comprising a variable region of an immunoglobulin gene, said method comprising the steps of:

a. providing genomic DNA comprising said immunoglobulin gene;

b. providing a first set of primers, each of which contains the sequence of a polymorphic variant of a segment of the 5′ untranslated region of said immunoglobulin gene;

c. providing a second set of primers, each of which contains the sequence of a polymorphic variant of a segment of the intron 3′ to the rearranged J region of said immunoglobulin gene;

d. carrying out a polymerase chain reaction with said genomic DNA and said first and said second sets of primers;

e. identifying a set of primers from said polymerase chain reaction that comprise sequences of said immunoglobulin gene; and

f. amplifying said immunoglobulin gene with said identified set of primers.

29. The method of claim 28, wherein said immunoglobulin gene is from hybridoma cell line HNK-20.

Fig. 1a)

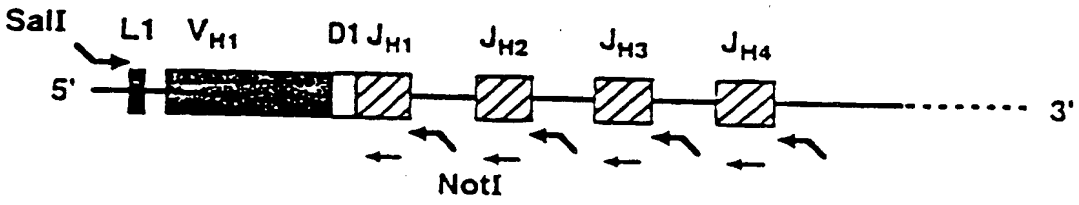


Fig. 1b)

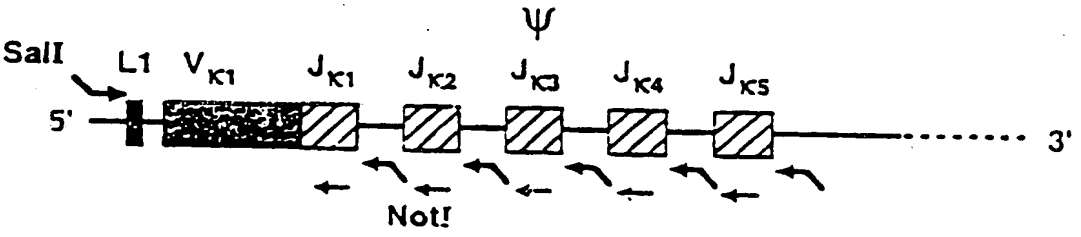


Fig. 1c)

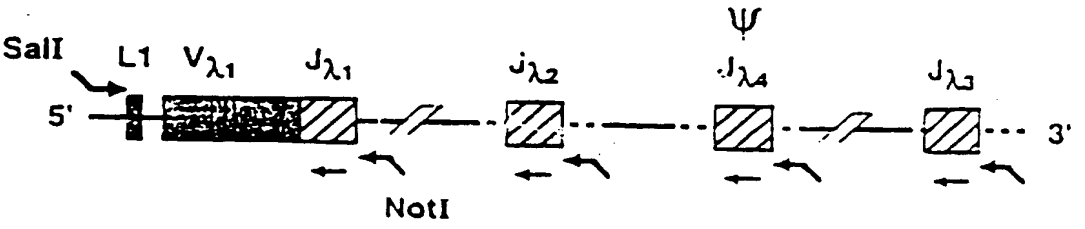


FIG. 2A

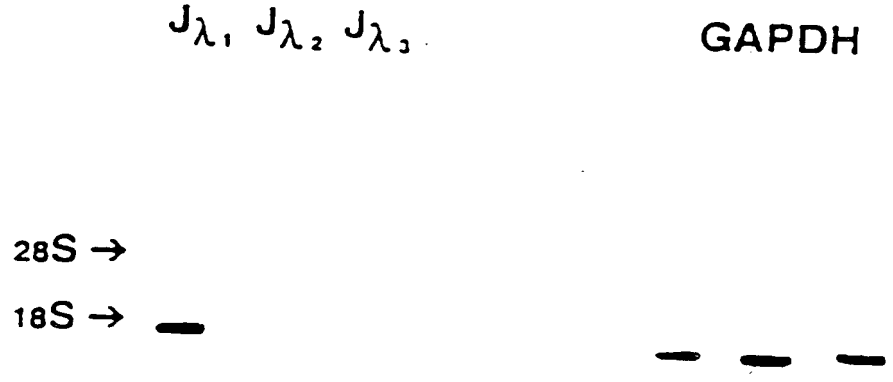
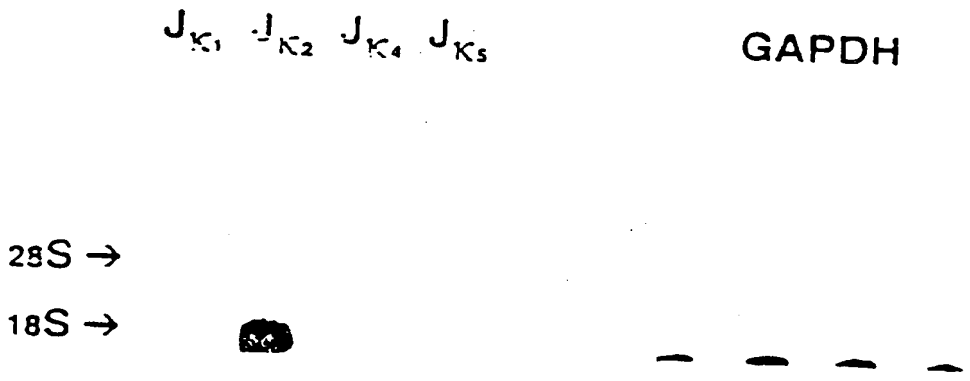


FIG. 2B



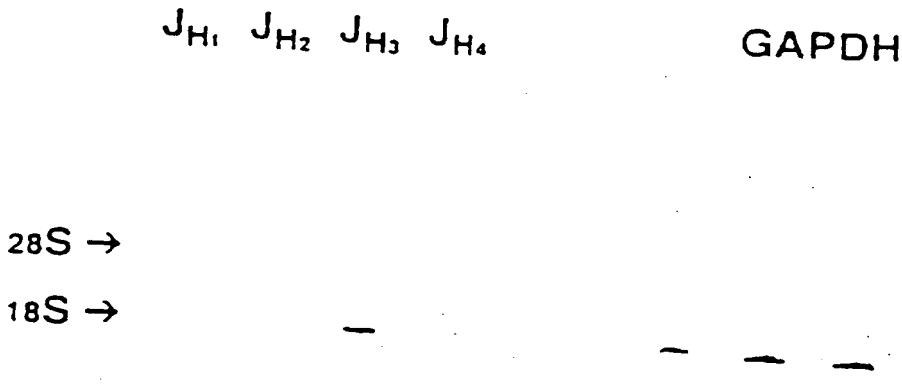


FIG. 3A

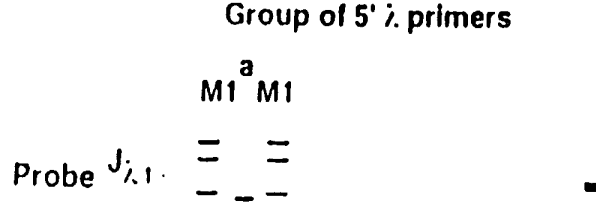


FIG. 3B

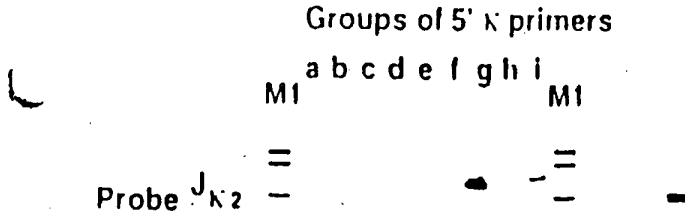


FIG. 3C

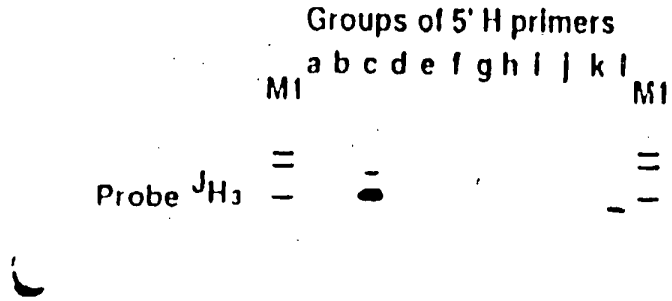


FIG. 3D

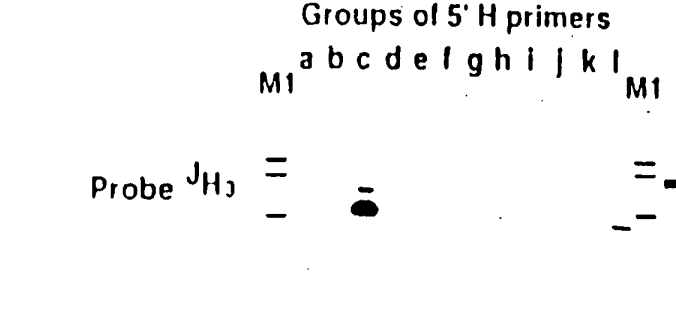


FIG. 3E

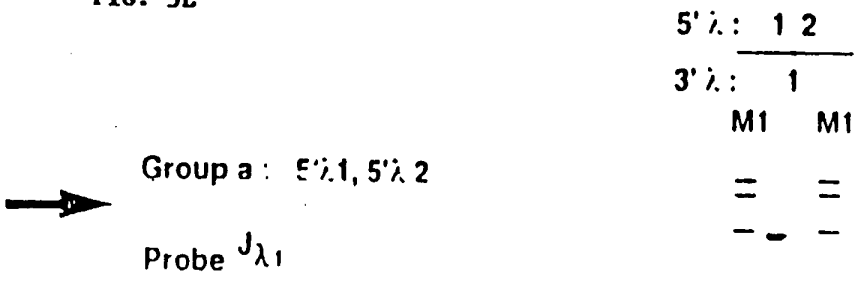


FIG. 3F

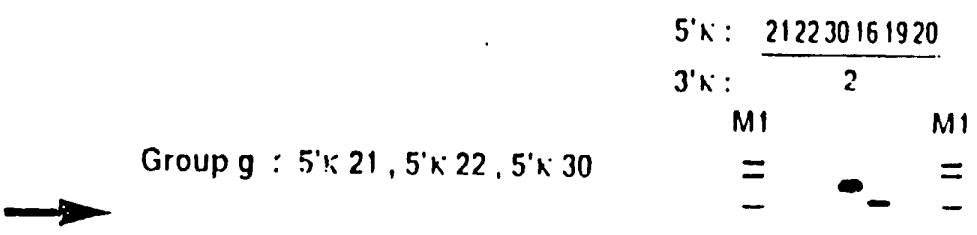


FIG. 3G

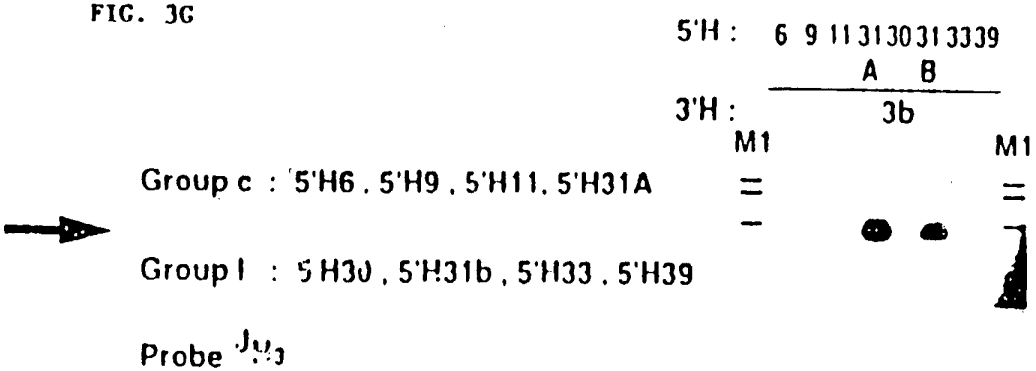


Fig. 4a

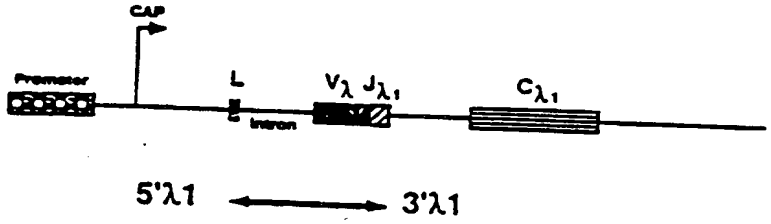


Fig. 4b

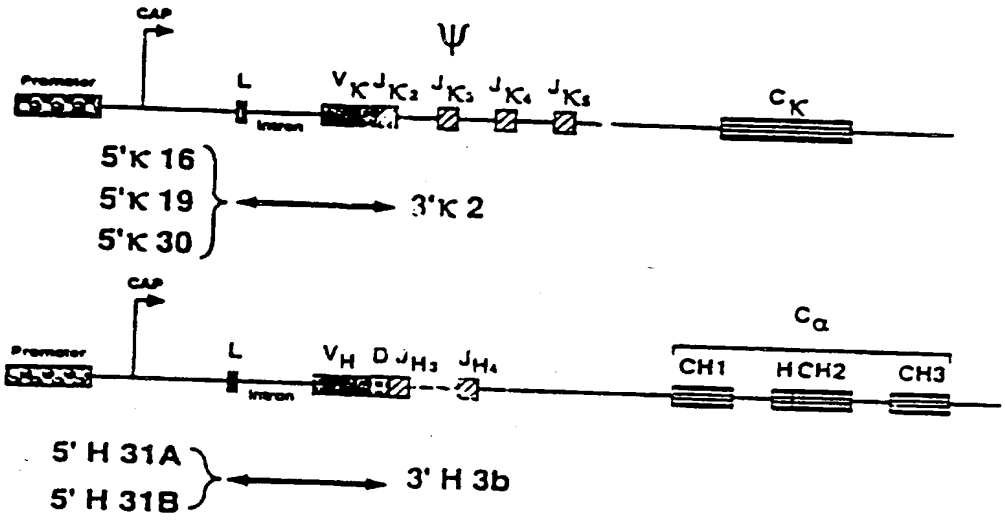


FIG. 4B

FIG. 5A

A

```
GATCGTCGACCGTGGTTTGTGAATTATG GCC TGG ATT TCA CTT ATA CTC TCT CTC   55   SEQ ID NO: 1
                             Met Ala Trp Ile Ser Leu Ile Leu Ser Leu   10   SEQ ID NO: 2

CTG GCT CTC AGC TCA G GTCAGCAGCCTTTCTACACTGCAGTGGTATGCAACAATGCGCAT  116
Leu Ala Leu Ser Ser G                                                15

CTTGTCTCTGATTTGCTACTGATGACTGGATTTCTCATCTGTTTGCAG GG GCC ATT TCC CAG  178
                                                  ly Ala Ile Ser Gln  20

GCT GTT GTG ACT CAG GAA TCT GCA CTC ACC ACA TCA CCT GGT GAA ACA GTC  229
Ala Val Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val  37

ACA CTC ACT TGT CGC TCA AGT ACT GGG GCT GTT ACA ACT AGT AAC TAT GCC  280
Thr Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala  54

AAC TGG GTC CAA GAA AAA CCA GAT CAT TTA TTC ACT GGT CTA ATA GGT GGT  331
Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly Leu Ile Gly Gly  71

ACC AAC AAC CGA GCT CCA GGT GTT CCT GCC AGA TTC TCA GGC TCC CTG ATT  382
Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu Ile  88

GGA GAC AAG GCT GCC CTC ACC ATC ACA GGG GCA CAG ACT GAG GAT GAG GCA  433
Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu Asp Glu Ala 105

ATA TAT TTC TGT GCT CTA TGG TAC AGC AAC CAT TGG GTG TTC GGT GGA GGA  484
Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn His Trp Val Phe Gly Gly Gly 122

ACC AAA CTG ACT GTC CTA G GTGAGTCACTGCTCCCTCCTTTGCGGCCGCTGAT        537
Thr Lys Leu Thr Val Leu                                             128
```

FIG. 5B

B

```
   GATCGTCGACGGACTCAGCATGGAGATG AGG ACC CCT GCT CAG TTT CTT GGA ATC   55   SEQ ID NO: 3
                              Met Arg Thr Pro Ala Gln Phe Leu Gly Ile   10   SEQ ID NO: 4

TTG TTG CTC TGG TTT CCA G GTAAAATGAACTAAAATGGGAATGTCACTGTGATTAGTGTTC 116
Leu Leu Leu Trp Phe Pro G                                             16

ATTGGCATTTGGGAGATTTTATCTTTTATGATGCTTACCTATGTAGATACTCATTATGTCTCCATTC 183

CTAG GT ATC AAA TGT GAC ATC AAG GTG ACC CAG TCT CCA TCT TCC ATG TAT  234
     ly Ile Lys Cys Asp Ile Lys Val Thr Gln Ser Pro Ser Ser Met Tyr   32

GCA TCT CTA GGA GAG AGA GTC ACT ATC ACT TGC AAG GCG AGT CAG GAC ATT 285
Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile  49

AAT AAC TAT TTA AAC TGG TTC CAG CAG AAA CCA GGT AAA TCT CCT AAG ACC 336
Asn Asn Tyr Leu Asn Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys Thr  66

CTG ATC TAT CGT GCA AAC AGA TTG GTA GAT GGG GTC CCA TCA AGG TTC AGT 387
Leu Ile Tyr Arg Ala Asn Arg Leu Leu Asp Gly Val Pro Ser Arg Phe Ser  83

GGC AGT GGA TCT GGG CAA GAT TAT TCT CTC ACC ATC AGC AGC CTG GAG TAT 438
Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Tyr 100

GAA GAT ATG GGA ATT TAT TAT TGT CTA CAG TTT GAC GAG TTT CCG TAC ACG 489
Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Phe Asp Glu Phe Pro Tyr Thr 117

TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA C GTAAGTAGTCTTCTCAACTCTTGCG 545
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys                              127

GCCGCTGAT                                                           554
```

FIG. 5C

c

```
GATCGTCGACTTCCAGCTCTCAGAGATG GAG ACA GAC ACA CTC CTG TTA TGG GTA   55   SEQ ID NO: 5
                             Met Glu Thr Asp Thr Leu Leu Leu Trp Val   10   SEQ ID NO: 6

CTG CTG CTC TGG GTT CCA G GTGAGAGTGCAGAGAAGTGTTGGATGCAACCTCTGTGGCCA  115
Leu Leu Leu Trp Val Pro G                                             16

TTATGATACTCCATGCCTCTCTGTTCTTGATCACTATAATTAGGGCATTTGTCACTGGTTTTAAGTT  182

TCCCCAGTCCCCTGAATTTTCCATTTCCTCAGAGTGATGTCCAAAATTCTTCTTAAAAATTTAAATC  249

AAAAGGTCCTCTGCTGTGAAGTCTTTTATACATATATAACAATAATCTTTGTGTTTATCATTCCAG  315

GT TCC ACT GGT GAC ATT GTG CTG ACA CAG TCT CCT GCT TCC TTA GCT GTA     365
ly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val     33

TCT CTG GGG CAG AGG GCC ACC ATC TCA TAC AGG GCC AGC AAA AGT GTC AGT   416
Ser Leu Gly Gln Arg Ala Thr Ile Ser Tyr Arg Ala Ser Lys Ser Val Ser    50

ACA TCT GGC TAT AGT TAT ATG CAC TGG AAC CAA CAG AAA CCA GGA CAG CCA   467
Thr Ser Gly Tyr Ser Tyr Met His Trp Asn Gln Gln Lys Pro Gly Gln Pro    67

CCC AGA CTC CTC ATC TAT CTT GTA TCC AAC CTA GAA TCT GGG GTC CCT GCC   518
Pro Arg Leu Leu Ile Tyr Leu Val Ser Asn Leu Glu Ser Gly Val Pro Ala    84

AGG TTC AGT GGC AGT GGG TCT GGG ACA GAC TTC ACC CTC AAC ATC CAT CCT   569
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His Pro   101

GTG GAG GAG GAG GAT GCT GCA ACC TAT TAC TGT CAG CAC ATT AGG GAG CTT   620
Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln His Ile Arg Glu Leu   118

ACA CGT TCG GAG GGG GGA CCA AGC TGG AAA TAA AAC GTAAGTAGTCTTCTCAACT  675
Thr Arg Ser Glu Gly Gly Pro Ser Trp Lys ***                           128

CTTGCGGCCGCTGAT                                                     690
```

FIG. 5D

D

```
GATCGTCGACCTCAAGGTCCTTACAATG AAA TGC AGC TCG GTC ATC TTC TTC CTG   55    SEQ ID NO: 7
                             Met Lys Cys Ser Trp Val Ile Phe Phe Leu   10    SEQ ID NO: 8

ATG GCA GTG GTT ACA G GTAAGGAGCCTCCCAAGTCCCAAACTTGAGCGGCCATACACTCTGT  116
Met Ala Val Val Thr G                                                  15

GACAGTGGCAGTCACTTTGCCTTTCTTTCTACAG GG GTC AAT TCA GAG GTT CAG CTG      173
                                   ly Val Asn Ser Glu Val Gln Leu      23

CAG CAG TCT GGG GCT GAG CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC    224
Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser    40

TGC AAA GCC TCT GGC TTC AAC ATT AAA GAC TAC TAT ATG TAC TGG GTA AAA    275
Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr Tyr Met Tyr Trp Val Lys    57

CAG AGG CCT GAA CAG GGC CTG GAG TGG ATT GGA TGG ATT GAT CCT GAA AAT    326
Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn    74

GGT AAT ACT GTT TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA    377
Gly Asn Thr Val Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala    91

GAC ACA TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG GCA TCT GAG    428
Asp Thr Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Ala Ser Glu   108

GAC ACT GCC GTC TAT TAC TGT GCT TAC TAC GGT ACT AGC TAC TGG TTT CCT    479
Asp Thr Ala Val Tyr Tyr Cys Ala Tyr Tyr Gly Thr Ser Tyr Trp Phe Pro   125

TAC TGG GGC CAA GGG ACC CTG GTC ACT GTC TCT GCA G GTGAGTCCTACCTTCTC   533
Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala                       137

CGCGGCCGCTGAT                                                        546
```

FIG. 6A

A) PCR primers for Vλ :

(a) 5'λ1 : GATCGTCGACCITGGTTTGTGAATTATG	SEQ ID NO: 9
(a) 5'λ2 : GATCGTCGACAGTAGTACCTGCATTATG	SEQ ID NO: 10
3'λ1 : GATCGCGGCCGCAAAGGAGGIAGGAGTIAC	SEQ ID NO: 11
3'λ2 : ATCAGCGGCCGCAAGAAGCATTAAAGCCAC	SEQ ID NO: 12
3'λ3 : ATCAGCGGCCGCAAGAAGCTTTGAAACTAC	SEQ ID NO: 13

FIG. 6B

B) PCR primers for Vκ :

(a) 5'κ1 : GATCGTCGACAAATTCAAAG/TACAA/CAAT	SEQ ID NO: 14
(f) 5'κ2 : GATCGTCGACAAGACTCAGCCTGACATG	SEQ ID NO: 15
(a) 5'κ3 : GATCGTCGACAAGTTCAAAGACAAAATG	SEQ ID NO: 16
(d) 5'κ4 : GATCGTCGACAGACTCAGCCTTGACATG	SEQ ID NO: 17
(h) 5'κ5 : GATCGTCGACAGCAGGGGGAGCAGGATG	SEQ ID NO: 18
(b) 5'κ6 : GATCGTCGACAGGGAAAGTTTGAAGATG	SEQ ID NO: 19
(d) 5'κ7 : GATCGTCGACATACATCAGACCAGCATG	SEQ ID NO: 20
(d) 5'κ8 : GATCGTCGACATCTAGC/TTCTCAGAGATG	SEQ ID NO: 21
(f) 5'κ9 : GATCGTCGACATGCATCACACCAGCATG	SEQ ID NO: 22
(d) 5'κ10: GATCGTCGACCACCAAGTTCTCAGAATG	SEQ ID NO: 23
(h) 5'κ11: GATCGTCGACCAGAGCAGCAGGGACATG	SEQ ID NO: 24
(h) 5'κ12: GATCGTCGACCAGGGACAAGTGGGAATG	SEQ ID NO: 25
(e) 5'κ13: GATCGTCGACCATTCAGAACTCAGCATG	SEQ ID NO: 26
(h) 5'κ14: GATCGTCGACGCGAGTCAGACCAGCATG	SEQ ID NO: 27
(b) 5'κ15: GATCGTCGACGGACACAGTTTAGATATG	SEQ ID NO: 28
(i) 5'κ16: GATCGTCGACGGACTCAGCATGGACATG	SEQ ID NO: 29
(e) 5'κ17: GATCGTCGACGGAGACGTTGTAGAAATG	SEQ ID NO: 30
(f) 5'κ18: GATCGTCGACGGATACACCATCAGCATG	SEQ ID NO: 31
(i) 5'κ19: GATCGTCGACGGCAAA/GGGCATCAAGATG	SEQ ID NO: 32
(i) 5'κ20: GATCGTCGACGGCAGG/TGGA/GAGCAAGAT	SEQ ID NO: 33
(g) 5'κ21: GATCGTCGACGGTCACAGCACAAACATG	SEQ ID NO: 34
(g) 5'κ22: GATCGTCGACGGTTGCCTCCTCAAAATG	SEQ ID NO: 35
(b) 5'κ23: GATCGTCGACGTTCATTTCCTCAAAATG	SEQ ID NO: 36
(a) 5'κ24: GATCGTCGACTATCAAGTTCTCAGAATG	SEQ ID NO: 37
(c) 5'κ25: GATCGTCGACTCTCAAGTTCTCAGAATG	SEQ ID NO: 38
(a) 5'κ26: GATCGTCGACTCTTGTGAATTAATCATG	SEQ ID NO: 39
(c) 5'κ27: GATCGTCGACTGAAAACACACAGACATG	SEQ ID NO: 40
(c) 5'κ28: GATCGTCGACTGATAAAGCCAAGGAATG	SEQ ID NO: 41
(c) 5'κ29: GATCGTCGACTGATCACACACAGA/TCATG	SEQ ID NO: 42
(g) 5'κ30: GATCGTCGACTTCCAGCTCTCAGAGATG	SEQ ID NO: 43
3'κ1 : ATCAGCGGCCGCAGAGAG/CTTTGGATTCTAC	SEQ ID NO: 44
3'κ2 : ATCAGCGGCCGCAAGAGTTGAGAAGACTAC	SEQ ID NO: 45
3'κ3 : ATCAGCGGCCGCAGTTGAGCAAAAATGTAC	SEQ ID NO: 46
3'κ4 : ATCAGCGGCCGCAAATGAGCAAAAA/GTCTAC	SEQ ID NO: 47
3 κ5 : ATCAGCGGCCGCAAGATGAGAAAAGTGTAC	SEQ ID NO: 48

FIG. 6C

C) PCR primers for VH :

(j) 5'H1 : GATCGTCGACACACAGACTCACACCATG	SEQ ID NO: 49
(g) 5'H2 : GATCGTCGACACACAGGACCTCACCATG	SEQ ID NO: 50
(j) 5'H3 : GATCGTCGACACACAGGATCTCACCATG	SEQ ID NO: 51
(g) 5'H4 : GATCGTCGACACACAGGGCATTGCCATG	SEQ ID NO: 52
(b) 5'H5 : GATCGTCGACACACTGACTCAAAACATG	SEQ ID NO: 53
(e) 5'H6 : GATCGTCGACACACTGACTCAAACCATG	SEQ ID NO: 54
(j) 5'H7 : GATCGTCGACACACTGACTCACACCATG	SEQ ID NO: 55
(j) 5'H8 : GATCGTCGACACACTGACTCCAACCATG	SEQ ID NO: 56
(e) 5'H9 : GATCGTCGACACACTGACTCTAACCATG	SEQ ID NO: 57
(k) 5'H10: GATCGTCGACACACTGACTCTCACCATG	SEQ ID NO: 58
(e) 5'H11: GATCGTCGACACACTGACTTTCACCATG	SEQ ID NO: 59
(h) 5'H12: GATCGTCGACACATAGACTCTAACCATG	SEQ ID NO: 60
(b) 5'H13: GATCGTCGACACATTGACTCAAACCATG	SEQ ID NO: 61
(g) 5'H14: GATCGTCGACAGCCTCCATCAGAGCATG	SEQ ID NO: 62
(e) 5'H15: GATCGTCGACAGCCTCCGTCAGAGCATG	SEQ ID NO: 63
(a) 5'H16: GATCGTCGACATTATAACATTGAACATG	SEQ ID NO: 64
(b) 5'H17: GATCGTCGACCAAGTCTTAGACATCATG	SEQ ID NO: 65
(k) 5'H18: GATCGTCGACCACACATCCCTTACCATG	SEQ ID NO: 66
(h) 5'H19: GATCGTCGACCACAGACACCTCACCATG	SEQ ID NO: 67
(e) 5'H20: GATCGTCGACCACAGACCACCTCACCATG	SEQ ID NO: 68
(k) 5'H21: GATCGTCGACCACAGACCTGTCAACATG	SEQ ID NO: 69
(h) 5'H22: GATCGTCGACCACAGACCTGTCACCATG	SEQ ID NO: 70
(e) 5'H23: GATCGTCGACCACGGAACCCTCACCATG	SEQ ID NO: 71
(f) 5'H24: GATCGTCGACCACGGACCCCTCACCATG	SEQ ID NO: 72
(f) 5'H25: GATCGTCGACCACGGACCCCTCACGATG	SEQ ID NO: 73
(k) 5'H26: GATCGTCGACCACTCGACTCTAACCATG	SEQ ID NO: 74
(h) 5'H27: GATCGTCGACCACTGGTGTGCAGTCATG	SEQ ID NO: 75
(a) 5'H28: GATCGTCGACCACTTCTTAGACATCATG	SEQ ID NO: 76
(f) 5'H29: GATCGTCGACCAGAGTCCACTCAGCCATG	SEQ ID NO: 77
(l) 5'H30: GATCGTCGACCCTGTCACTGACTTCATG	SEQ ID NO: 78
(c) 5'H31a:GATCGTCGACCTCAAGGTCCTTACAATG	SEQ ID NO: 79
(t) 5'H31b:GATCGTCGACCTCCAGGTCCTTACAATG	SEQ ID NO: 80
(i) 5'H32: GATCGTCGACCTCAGTCCTGTCACCATG	SEQ ID NO: 81
(l) 5'H33: GATCGTCGACCTCAGTCCTGTCACTATG	SEQ ID NO: 82
(i) 5'H34: GATCGTCGACGCAGAGGACCTCACAATG	SEQ ID NO: 83
(d) 5'H35: GATCGTCGACGCCTTTACAGACTTGATG	SEQ ID NO: 84
(f) 5'H36: GATCGTCGACGGACCTCACCATGGGATG	SEQ ID NO: 85
(i) 5'H37: GATCGTCGACGGGTGTTGCCTAAGGATG	SEQ ID NO: 86
(d) 5'H38: GATCGTCGACGGTGTA/TGCCTAAAAGATG	SEQ ID NO: 87
(l) 5'H39: GATCGTCGACGGTGTTGCCTAAAGGATG	SEQ ID NO: 88
(a) 5'H40: GATCGTCGACGTTGTAGCCTAAAAGATG	SEQ ID NO: 89
(d) 5'H41: GATCGTCGACTCAGTCCTTGTCACTATG	SEQ ID NO: 90
3'H1 : ATCAGCGGCCGCAAAGAAAAAAGCCAGCTTAC	SEQ ID NO: 91
3'H2 : ATCAGCGGCCGCGAGGTTT/GTAAGGACTCAC	SEQ ID NO: 92
3'H3a: ATCAGCGGCCGCGGAGAAA/GTTAGGACTCAC	SEQ ID NO: 93
3'H3b: ATCAGCGGCCGCGGAGAAGT/GTAGGACTCAC	SEQ ID NO: 94
3'H4 : ATCAGCGGCCGCTGGAGAGGCCATTCTTAC	SEQ ID NO: 95

FIG. 6D

D) Jλ oligo probes :

Jλ1 : GTCAGTTTGGTTCCTCCAC	SEQ ID NO: 96
Jλ2 : GTGACCTTGGTTCCACCGC	SEQ ID NO: 97
Jλ3 : GTGACCTTGGTTCCACTGC	SEQ ID NO: 98
Jλ4 : GTCAATCTGGTTCCACCTC	SEQ ID NO: 99

FIG. 6E

E) Jκ oligo probes :

Jκ1 : GTGCCTCCACCGAACGTCC	SEQ ID NO: 100
Jκ2 : GTCCCCCCTCCGAACGTGT	SEQ ID NO: 101
Jκ3 : GTCCCATCACTGAATGTGA	SEQ ID NO: 102
Jκ4 : GTCCCCGAGCCGAACGTGA	SEQ ID NO: 103
Jκ5 : GTCCCAGCACCGAACGTGA	SEQ ID NO: 104

FIG. 6F

F) JH oligo probes :

JH1 : GACCGTGGTCCCTGCGCCC	SEQ ID NO: 105
JH2 : GAGAGTGGTGCCTTGGCCC	SEQ ID NO: 106
JH3 : GACCAGAGTCCCTTGGCCC	SEQ ID NO: 107
JH4 : GACTGAGGTTCCTTGACCC	SEQ ID NO: 108

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/15716

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.53; 435/320.2, 240.2, 240.42, 91.2, 70.21; 530/387.3, 388.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Virology, Volume 63, Number 2, issued February 1994, Garcia-Barreno et al., "Marked Differences in the Antigenic Structure of Human Respiratory Syncytial Virus F and G Glycoproteins", pages 925-932, especially figures 3 and 4 on page 928.	1-29
Y	Proceedings of the National Academy of Sciences USA, Volume 86, issued May 1989, Orlandi et al., "Cloning Immunoglobulin Variable Domains for Expression by the Polymerase Chain Reaction", pages 3833-3837, see entire article.	1-29

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
15 MARCH 1996

Date of mailing of the international search report
25 MAR 1996

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/15716

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Bio/Technology, Volume 9, issued March 1991, Tempest et al., "Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection *In Vivo*", pages 266-271, see entire article.	1-29
Y	Journal Hyg. Camb., Volume 68, issued 1970, Scott et al., "Respiratory Syncytial Virus Neutralizing Activity in Nasopharyngeal Secretions", pages 581-588, see entire document.	1-29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/15716

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

C07H 15/12; C12N 15/00, 15/13, 5/10, 1/20; C07K 16/08; C12P 19/34, 21/08

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/23.53; 435/320.2, 240.2, 240.42, 91.2, 70.21; 530/387.3, 388.3